Media Peternakan, April 2012, pp. 67-72
EISSN 2087-4634
Accredited by DGHE No: 66b/DIKTI/Kep/2011

Online version:
http://medpet.journal.ipb.ac.id/
DOI: 10.5398/medpet.2012.35.1.67

The Role of Arbuscular Mycorrhizal Fungi in Enhancing Productivity, Nutritional Quality, and Drought Tolerance Mechanism of *Stylosanthes seabrana*

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(Received 28-10-2011; accepted 15-03-2012)

ABSTRAK

Fungi mikoriza arbuskular (FMA) adalah asosiasi simbiosis antara akar tanaman dan fungi. Peran utama FMA adalah untuk meningkatkan serapan hara dan air oleh tanaman inang. Tujuan dari penelitian ini untuk mempelajari peran FMA dalam meningkatkan produktivitas, kualitas nutrisi dan mekanisme toleransi dari *Stylosanthes seabrana* dalam kondisi kekeringan. Penelitian ini menggunakan rancangan acak lengkap dengan empat perlakuan: A0 (tanpa FMA), A1 (tanpa FMA dengan kekeringan), A2 (dengan FMA) dan A3 (dengan FMA dengan kekeringan) pada tanaman leguminosa *S.s seabrana*. Parameter yang diamati adalah kandungan air tanah, potensial air daun, kandungan air relatif daun (RWC), berat kering tajuk dan akar, prolin, gula terlarut, protein kasar, produksi gas, dan kecernaan bahan organik. Data dianalisis dengan analisis varians (ANOVA) dan perbedaan antara perlakuan dianalisis dengan uji Duncan. Hasil penelitian menunjukkan bahwa inokulasi FMA meningkatkan berat kering tajuk dan akar, protein kasar, produksi gas, kecernaan bahan organik, akan tetapi menurunkan prolin dan gula larut secara signifikan (P<0,05). Kekeringan dapat menurunkan kadar air tanah, potensial air daun, berat kering tajuk dan akar, protein kasar, produksi gas, kecernaan bahan organik, akan tetapi terjadi peningkatan prolin dan gula larut secara signifikan (P<0,05). Mekanisme toleransi kekeringan pada *S. seabrana* melalui akumulasi prolin dan gula terlarut.

Kata kunci: Fungi Mikoriza Arbuskula, Stylosanthes seabrana, kekeringan, prolin, gula terlarut

ABSTRACT

Arbuscular Mycorrhizal Fungi (AMF) is a symbiotic association between plant roots and fungi. Their major role is to enhance nutrient and water uptake by the host plants. The objective of this research was to study the role of AMF in enhancing productivity, nutritional quality and tolerance mechanism of *Stylosanthes seabrana* in drought conditions. This research used a completely randomized design with four treatments: A0 (without AMF), A1 (without AMF in drought), A2 (with AMF), and A3 (with AMF in drought) in *S. seabrana*. Parameters observed were the soil moisture content, water potential of shoot, relative water content of leaf (RWC), root length, shoot and root dry weight, proline, soluble sugars, crude protein, gas production, and digestibility of organic matter. The data were analyzed with analysis of variance (ANOVA) and the differences between treatments were analyzed with Duncan Multiple Range test. Results showed that inoculation of AMF could enhance leaf water potential, shoot and root dry weight, crude protein, gas production, digestibility of organic matter, but decreased proline and soluble sugars significantly (P<0.05). Drought reduced soil moisture, leaf water potential, shoot and root dry weight, crude protein, gas production, digestibility of organic matter, but enhanced proline and soluble sugars significantly (P<0.05). The drought tolerance mechanism of *S. seabrana* seems likely through accumulating organic osmolytes such as prolines and soluble sugars.

Key words: Arbuscular Mycorrhizal Fungi, Stylosanthes seabrana, drought, proline, soluble sugars

INTRODUCTION

Plants in nature are continuously exposed to several biotic and abiotic stresses, water deprivation being one of the most common problem. Dry land for crop production have been estimated to cover 28% of the Earth's land surface (Bray, 2004). Nevertheless, plants have developed several physiological, biochemical, and molecular mechanisms in order to cope with drought stress. Plant responses to water stress include morphological and biochemical changes that lead to acclimation and then to functional damage and the loss of plant parts

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(Chaves *et al*., 2003). During the acclimation phase, water stress typically results in slower growth rates because of the inhibition of cell expansion, the reduction in carbon assimilation and the resultant effect on carbon partitioning. In crops such as common beans, these reductions can impact directly on the abscission rate of flowers, a major determinant of yield (Clements & Atkins, 2001).

Arbuscular Mycorrhizal Fungi (AMF) is a symbiotic association between plant roots and fungi. Their major role is to enhance nutrient and water uptake by the host plant. Water stress is one of the most important environmental factors that regulate plant growth and development, and limit plant production. Plants can respond and adapt to water stress by altering their cellular metabolism and invoking various defence mechanisms (Smith & Read, 2008). The beneficial effect of AMF symbiosis under drought-stress conditions has been studied largely at the physiological level including regulation of transpiration rate or increasing root water absorption (Auge, 2001; Auge, 2004). More recently, it has also been noted that, under drought-stress conditions, AMF and without AMF plants regulate differently the expression of several stress related genes in root tissues (Ruiz-Lozano *et al*., 2006). Several studies on the topic have demonstrated that the contribution of the AMF symbiosis to plant drought tolerance results from a combination of physical, nutritional, physiological, and cellular effects (Ruiz-Lozano, 2003). The objective of this research was to study the role of AMF in enhancing productivity, nutritional quality and drought tolerance mechanism of *Stylosanthes seabrana.*

MATERIALS AND METHODS

The material used in this study was *Stylosanthes seabrana* resulted from selection of about 30 species forages on preliminary research. Fiber pots (d= 20 cm, h= 100 cm), mycofer, growing media in the form of soil and manure, WP4 potentiometer, coolbox were used in the experiment.

This research used a completely randomized design with four treatments: A0 (without AMF), A1 (without AMF in drought), A2 (with AMF), and A3 (with AMF in drought) and four replications and each replication consist of 2 unit plants. The research was conducted in the Agrostology, and the Dairy Nutrition Laboratory, Faculty of Animal Science, Bogor Agricultural University. Stress parameter measurement was observed in Stress Physiology Laboratory at the Indonesian Institute of Sciences, Cibinong.

The parameters observed were: a) soil moisture content, the water content of soil at a depth of 20 cm calculated using reflectometry. Soil moisture content from the beginning to the end of treatment was measured 9 times, within 4-days interval from the beginning of treatment (day 0, 4, 8, 12, 16, 20, 24, 28, and 32) for the treatment of drought stress, b) Leaf water potential measurements were performed 9 times during treatment, within 8-days interval from the beginning of treatment (day 0, 8, 16, 24, 32, 40, and 48). Leaf water potential in the non-stress and stress conditions was tested using the WP4, c) Relative water content of shoot (RWC)= (fresh weight-dry weight)/(turgor weight- dry weight) x 100%, d) Root length, the starting point of the root length was measured in the plants that will be transferred to pots and growing media treatments at harvest in the day 32^{nd}, e) Shoot and root dry weight measurements were performed at the end of the harvesting, by weighing fresh, and then dried at oven 70 °C until reaching constant weight, f) Proline (Bates 1973), g) Soluble sugars (modified by Buysse & Merckx 1993), soluble sugars were analysed by 0.1 ml of the alcoholic extract reacting with 3 ml freshly prepared anthrone (200 mg anthrone + 100 ml 72% (w:w) H_2SO_4) and placed in a boiling water bath for 10 min according to Irigoyen *et al.* (1992). After cooling, the absorbance at 620 nm was determined in a Shimadzu UV-1603 spectrophotometer. The callibration curve was made using glucose in the range of 20–400 μg ml^{-1}. h) Crude protein (Kjeldhal method), i) Gas production (Close & Menke, 1986), j) Digestibility of organic matter (Tilley & Terry, 1963). The data were analyzed with analysis of variance (ANOVA) and the differences between treatments were analyzed with Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Soil Moisture

Water is needed by the plants as a solvent, nutrient transport, maintain cell turgidity, raw materials of photosynthesis and nearly 70% of the plant is water. Plants need adequate water resources for the process of growth and development. If there is water shortage, it would be a direct result of inhibition of the growth process, metabolic disturbances and eventually cause a decreased in crop production (Taiz & Zeiger, 2002). Figure 1 shows the effect of treatments on changes in soil moisture content until day 48^{th}. Drought on day 40^{th} was significantly ($P<0.05$) decreased soil moisture. Control (A0) and the addition of AMF (A2) was significantly different ($P<0.05$) with drought-stressed (A1) and A3 (AMF in Drought). The addition of AMF in drought conditions (A3) did not show significant differences, but the trend of soil moisture content was higher than the drought-stressed (A1).

The soil moisture declined progressively during 48 d in drought condition. However, without AMF plants, the soil moisture content decreased at a faster rate than AMF plants. This indicated that AMF plants extracted soil water more slowly and developed less-intensive stress than those without AMF plants.

The soil moisture showed a significant difference (Figure 1). Treatment of drought stress by delaying the addition of water in to the medium caused a decrease in soil water content and leaf water potential. The soil moisture content changes in the treatment of drought stress was at average value of 22.40%, while under well-watered had an average value of 33.09%, so it can be said that a decline in soil water content at 10.7% compared to those of well-watered plants. Water content in soil describes the amount of available water resources, which is absorbed by plants to grow and drought causes the water becomes unavailable, and the plant suffers from wilt (Karti, 2004).

Leaf Water Potential

The leaf water potential determined at the end of the drought period was similar in plants treated with and without AMF cultivated under well-watered conditions (Figure 2). Drought stress decreased leaf water potential but the decrease was larger in plants without AMF (A1) about -3.65 Mpa than in AMF plants (A3) about -2.29 Mpa. The time-course of leaf water potential during the entire drought period showed a similar pattern for treatment with AMF and without AMF plants, both under well-watered and under drought-stress conditions, without AMF in drought plants always exhibited lower leaf water potential than plants with AMF. Porcel & Ruiz-Lozano (2004) reported that the leaf water potential was also higher in stressed AMF plants (−1.9 MPa) than plants without AMF (−2.5 MPa). Querejeta *et al*. (2003, 2006) reported that in a field study on mycorrhizae and water relations, mycorrhizae enhanced the plant water through flow at the critical point when leaf water potential ranged from –2 to –3.5 MPa, and soil water potential in the rooting zone was between –1.5 and –2 MPa. AM F could enhance water and related to the external hyphal matrix.

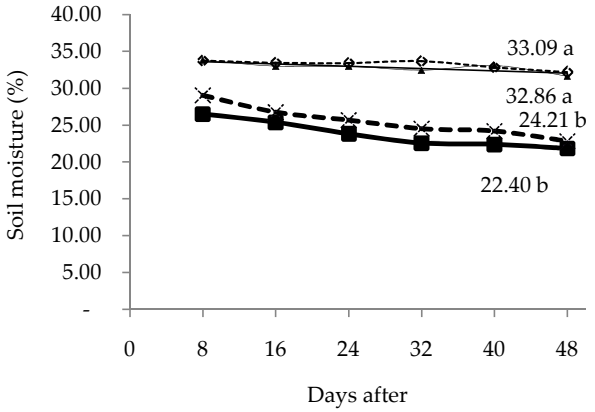

Figure 1. Change of soil moisture during treatment, control/A0 (-○-), drought/A1 (-■-), arbuscular mycorrhizal fungi (AMF) /A2 (-▲-), AMF & drought /A3 (-x-).

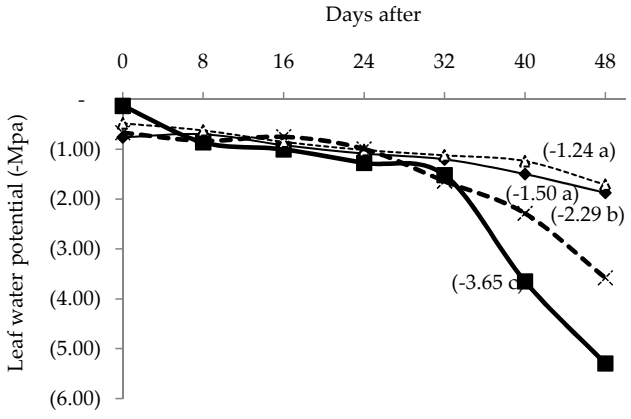

Figure 2. Change of leaf water potential during treatment, control/A0 (-♦-), drought/A1 (-■-), arbuscular mycorrhizal fungi (AMF)/A2 (-Δ-), AMF & drought /A3 (-x-).

Leaf Relative Water Content

Under well-watered with AMF or without AMF significantly different (P<0.05) with drought stress for leaf relative water content (Figure 3). Under well-watered and drought-stressed leaf relative water content of AMF plants were higher than plants without AMF. The leaf relative water content in drought-stressed AMF plants or without AMF plants was decreasing, caused by soil water content and leaf water potential declined (Figure 1 and 2). Jianping & Bughrara (2008) reported that drought-stress treatment had a significant (P<0.001) effect on Leaf Water Content (LWC) of the grasses. The LWC under well-watered plants remained constant at about 87.7% during the whole experimental periode. In plants subjected to drought, LWC decreased differently among the four grasses. Porcel & Ruiz-Lozano (2004) reported that LWC were significantly higher in AMF plants than plants without AMF. In addition, previous studies with soybean plants subjected to a similar drought-stress level have shown that AMF plants exhibited higher leaf water potential than plants without AMF. Allen (2006) reported that fungal hyphae have an additional architectural feature that also makes mycorrhizae important to water dynamics. Individual hyphae will wrap around each other, forming a space between linear, hydrophobic surfaces. More primitive fungi, such as AMF hyphae, can form wrapping "networks" of two to five hyphae extending a few centimeters into the soil in some complex basidiomycetes, these fungi can form highly structured "chords" that have vessel elements that are known to rapidly transport water and nutrients.

Shoot and Root Dry Weight

Under well-watered conditions, shoot dry weight of AMF plant (A2) were higher than those without AMF (A0) *S. seabrana* plants (Table 1). AMF plants showed an increase shoot dry weight about 30%. Drought stress decreased plant growth in both treatments (50% in plants without AMF and 40% in AMF plants). Under well-watered conditions, root dry weight of AMF (A2)

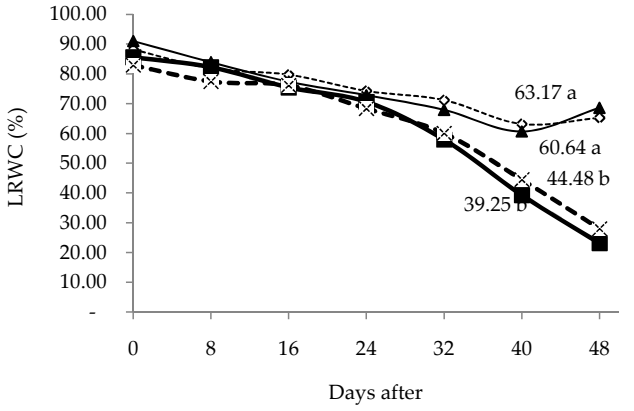

Figure 3. Change of leaf relative water content during treatment, control/A0 (-○-), drought/A1 (-■-), arbuscular mycorrhizal fungi (AMF)/A2 (-▲-), AMF & drought /A3 (-x-).

was higher than those without AMF (A0) at *S seabrana* plants (Table 1). AMF plants showed an increased in root dry weight about 20%. Drought stress decreased plant growth in both treatments (20% in plants without AMF and 10% in AMF plants). AMF in drought plants showed increased in roots dry weight about 10% as compared with AMF plants.

Decreasing of shoot and root dry weight in drought conditions caused by a decreasing in soil moisture content, lowering the water potential and leaf relative water content (Figure 1, 2, and 3). The Ribulose-1,5-*bis*phosphate (RuBP) in the leaves decreased with drought stress, it could contribute to the drought-induced decrease in photosynthesis (Taiz & Zeiger, 2002).Water deficit has profound effects on crop production. Plants with an optimum water supply experience transient water-shortage periods, where water absorption cannot compensate for water loss by transpiration. Arbuscular mycorrhizal symbiosis has been shown to increase plant tolerance to water deficit, although the exact mechanisms involved are still a matter of debate (Auge, 2001; Ruiz-Lozano, 2003). AMF plants showed a higher tolerance to the drought stress imposed (only for 48 d) than plants without AMF, as shown by their enhanced shoot biomass production (27%), higher leaf water potential under such conditions. Karti (2004) reported that the interaction effect between AMF and water treatment were not significantly different. The plant growth and production were decreased with lower water content of soil and AMF plant was better than plants without AMF..

Drought Tolerance Mechanism

Under well-watered plants, there were not significant differences on proline content in AMF and without AMF plants. Under drought-stressed, plants were significantly difference ($P<0.05$) in proline content of AMF plants and plants without AMF. Accumulation of proline increased considerably in leaf as a consequence of drought stress and plants without AMF accumulated 58% more proline than AMF plants under drought-stressed (Figure 4).

Under well-watered plants, total soluble sugars in shoots were higher in plants without AMF than AMF plants (Figure 5). Drought stress increased sugar accumulation in both treatments, and significantly difference ($P<0.05$). Drought increased the sugar content in plants without AMF by 116%, while AMF plants showed sugar content similar to well-watered conditions. Drought-stressed plants have been shown to accumulate organic osmolytes such as sugar and amino acids (proline) that are known to contribute to the host-plant tolerance under water-deficit conditions (Whittaker *et al.*, 2007). The enhanced sugar content in AMF roots under well-watered conditions may be due to the sink effect of the mycorrhizal fungus demanding sugars from shoot tissues. Under drought the sugar content in roots was similar in both treatments, suggesting that osmotic adjustment occurred. In contrast, in shoots the sugar content of droughted AMF plants was considerably lower than in plants without AMF. Schellembaum *et al.* (1998) suggested that the AMF can be a strong competitor for root-allocated carbon under conditions of limiting photosynthesis and the lower hexose accumulation in leaves of mycorrhizal plants in drought could be due to a lower availability of photosynthates for storage in these tissues. However, another explanation is also possible, that AMF shoots were less strained by drought than those without AMF. The lower accumulation of compatible solutes may indicate that the plants more

Table 1. Effect of treatments on shoot and root dry weight (g/pot)

Variables	Treatments			
	A0	A1	A2	A3
Shoot dry weight	49.7±5.1^{b}	28.1±4.0^{c}	61.4±5.7^{a}	32.6±8.0^{c}
Root dry weight	4.3±0.7^{ab}	3.6±0.7^{b}	5.1±0.3^{a}	3.9±1.5^{ab}

Note: Means in the same row with different superscript differ significantly ($P<0.05$). Treatments: A0= without arbuscular mycorrhizal fungi (AMF), A1= without AMF in drought, A2= with AMF, and A3= with AMF in drought.

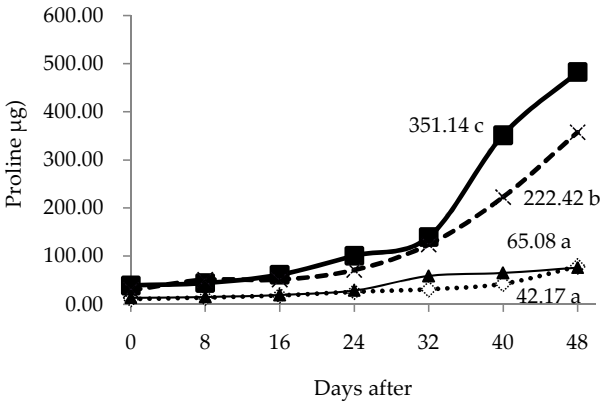

Figure 4. Change of proline content during treatment, control/ A0 (-◊-), drought/A1 (-■-), arbuscular mycorrhizal fungi (AMF)/A2 (-▲-), AMF & drought /A3 (-x-).

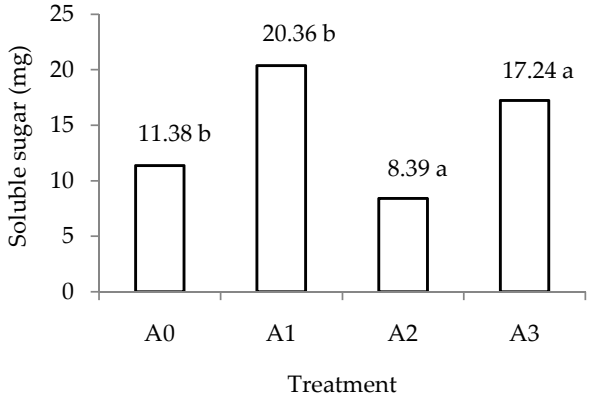

Figure 5. Soluble sugar content of each treatment (mg/g leaf dry weight), A0= control, A1= drought, A2= arbuscular mycorrhizal fungi (AMF), A3= AMF & drought.

successfully avoided drought stress (Augé, 2001). In fact, proline, the other osmoregulator measured in this study, also accumulated less in shoots of AMF plants than in plants without AMF.

Leaf water potential was higher in AMF drought plants (–2.29 MPa) than in plants without AMF (–3.65 MPa). The accumulation of proline and total soluble sugar in shoots as an osmotic mechanism is to maintain a favorable gradient for water entrance into the roots and to a lower stress injury in the plant. In addition to acting as an osmoprotectant, proline and soluble sugar also serve as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger, as a solute that protects macromolecules against denaturation, and as a means of reducing acidity in the cell (Kishor *et al.*, 1995). Accumulation of proline increased considerably in roots as a consequence of drought stress and AMF plants accumulated 14% more proline in roots than plants without AMF. In shoots, drought stress also induced the accumulation of proline. However, in such plant tissue, AMF plants accumulated 39% less proline than plants without AMF (Porcel & Ruiz-Lozano, 2004).

In addition to the above-discussed drought-tolerance mechanisms, the AMF contribution to plant drought tolerance might also have occurred through drought avoidance mechanisms such as hyphal water uptake (Marulanda *et al.*, 2003) or increased water uptake related to mycorrhizal changes in root morphology or soil structure (Auge *et al.*, 2001*a*). Such mycorrhizal effects could allow plants to remain more hydrated than plants without AMF as soil dries (Auge *et al.*, 2001b). Data from the present study, such as the higher mid-day leaf water potential in AMF than in plants without AMF, the lower accumulation of soluble sugar and proline in shoots of AMF than in plants without AMF.

Root and shoot tissues are influenced by AMF symbiosis by means of drought-avoidance and drought-tolerance mechanisms. It seems that first the AMF symbiosis enhances osmotic adjustment in roots which could contribute to maintain favorable gradient to the water passing from soil into the roots. The leaf water potential in AMF plants was higher than plants without AMF during drought and keeps the plants protected against oxidative stress, and these accumulative effects increase the plant drought tolerance. Mycorrhizal colonization and drought interact in modifying free amino acids and sugar pools in roots and a greater osmotic adjustment has also been reported in leaves of mycorrhizal plants than in plants without mycorrhizal during a lethal drought period (Kubikova *et al.*, 2001).

Studies *in Vitro* Quality of Organic Material

The drought stress treatment greatly affected the rumen fermentation. Total gas production in drought plants were lower (P<0.01) than the watered plants, that means low fermentation process (Figure 6). The gas production showed a very significance difference (P<0.01) among the treatments. AMF treatment (A1) produced the highest yield of gas (45.31 ml/200 mg DM), whereas the lowest gas production in the drought treatment was 29.77 ml/200 mg DM. There was an increase of 4.14% of gas production for AMF treatment in drought stress conditions (A2) compared to AMF (A3). It has been reported that some tropical browse plants, without water stress, such as *M. oleifera, G. sepium, C. calothyrrus* and *L. leucocephala* produced high gas production i.e., 140, 125, 120, 115 ml/200 mg DM, respectively. High gas production on those forages was related to nutrient content, rumen microbial metabolism and percentage of digestibility (Astuti *et al.*, 2011).

Average digestibility of organic matter showed that the AMF treatment (A1) had the highest value, whereas the drought treatment (A1) was low and no different with A3. This suggests that the apparent role of AMF on the condition of soil adequate water (flushing) availability which had not happen in drought stress conditions. It has been well documented that drought **stress**es are responsible for the increase in cell wall lignifications which would be associated with decreased plant growth, nutrient content, and digestibility (Guenni *et al.*, 2002). Bok-Rye Lee (2007) reported that the lignification process and its physiological significance under drought-stressed conditions. The changes of enzymes responsible for lignification and the related physiological parameters were determined in white clover (*Trifolium repens* L.) leaves during 28 d of water deficit treatment. Water deficit gradually decreased leaf water potential (Ψ_w) to −2.33 MPa at day 28. The reduction of leaf biomass occurred from 21 d of water deficit treatment when Ψ_w was −2.27 MPa or less, and it was paralel with the increase of lipid peroxidation and lignin content. Legumes without stress treatment had high dry matter digestibility.

Crude protein content of legumes *S. seabrana* showed different results (P<0.01) among the four treatments. The best treatment sequence was A2, A0, A3 and A1, respectively. This suggests that drought stress treatments influenced the reduction levels of crude protein of plants and AMF in drought stress conditions can increase levels of crude protein (A3) when compared with the drought treatment (A1). Giving AMF (A2) may increase crude protein when compared with the control (A0). Protein degradation has close correlation with dry matter degradability in the rumen (Rusdi *et al.*, 2008).

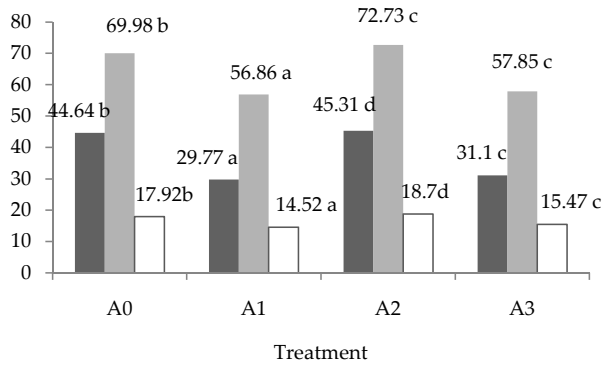

Figure 6. Means of gas production, organic matter digestibility, and crude protein of each treatments. ■= Gas production (ml/200 mg DM), ▩= Organic matter digestibility (%), □= Crude protein (%). A0= control, A1= drought, A2= AMF, A3= AMF & drought.

CONCLUSION

Drought stress can reduce soil moisture, leaf water potential, shoot and root dry weight, crude protein, gas production and digestibility of organic matter and enhanced proline and soluble sugar significantly. Inoculation of AMF can enhance leaf water potential, shoot and root dry weight, crude protein, gas production and digestibility of organic matter and decreased proline and soluble sugar significantly under drought stress. The drought tolerance mechanism of *S. seabrana* was possibly by accumulating organic osmolytes such as prolines and soluble sugars.

ACKNOWLEDGEMENT

Thanks to the Ministry of Research and Technology for applied incentive grants funding.

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